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TITLE: Combinational Targeting of Prostate Carcinoma Cells and Tumors Associated Pericytes with Antibody Based Immunotherapy and Metronomic Chemotherapy

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14. ABSTRACT The hybridoma secreting the HMW-MAA-specific mAb 225.28 which is used for immuno prevention of prostate carcinoma and the hybridoma secreting the isotype matched mAb F3C25 have been tested for activity. Ascitis has been prepared and monoclonal antibodies have been purified and monitored for purity and activity. The colony of TRAMP mice has been expanded to test the efficacy of mAb 225.28 plus cyclophosphamide metronomic therapy in the inhibition of progression of prostate cancer. Sixty-four TRAMP mice have been enrolled in the combinatorial treatment schedule. Animals are being screened 2 times a week for palpable tumors.					
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Table of Contents

Introduction.....	4.
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	none
Appendices.....	6

The hybridoma which secretes the HMW-MAA-specific mAb 225.28 has been defrosted and grown in culture. The spent medium has been harvested and tested for reactivity in a binding assay with the parental human melanoma M14 cell line, which does not express HMW-MAA, and with the HMW-MAA cDNA transfected M14 cell line which expresses HMW-MAA. The latter cell line is referred to as M-14/HMW-MAA. The spent medium was found to be active.

The hybridoma was injected into BALB/c mice to produce ascitis. Ninety ml of ascitis have been produced. The ascitis has been tested for activity with the M14/HMW-MAA and the M14 melanoma cell lines in a binding assay. One hundred and forty mg of mAb 225.28 have been purified from ascitis by sequential precipitation with caprylic acid and with ammonium sulphate. The purity of the antibody preparation has been monitored by SDS-PAGE analysis (Fig.1). The activity of the antibody preparation has been monitored by testing in ELISA with the cultured human melanoma cell line Colo 38 which expresses HMW-MAA and with the cultured human B lymphoid cell line LG-2 which does not express the HMW-MAA (Fig. 2).

Additionally, the hybridoma F3C25 has also been injected into SCID mice to produce ascitis. This hybridoma secretes an IgG2a mAb to be used as an isotype matched control for the in vivo studies in TRAMP mice. One hundred and twenty ml of ascitis have been produced. One hundred and forty mg of monoclonal antibody have been purified by sequential precipitation with ammonium sulphate and caprylic acid (Fig. 2).

To test the efficacy of mAb 225.28 plus cyclophosphamide (CTX) metronomic therapy in inhibition of the progression of prostate cancer in TRAMP mice, the TRAMP breeding colony has been expanded to 20 breeding cages comprised of 2 females and 1 male. Currently we have 64 TRAMP mice that have been enrolled in the combinatorial treatment schedule. TRAMP male mice were started on therapy at 10 weeks-of-age and are maintained on treatment until palpable tumors form. The study was initiated the first week of October and animals were assigned to one of three combinatorial treatments. Cohort 1 (n=21) is receiving AN2 mAb treatment twice weekly ip (100 µg/mouse/injection) in combination with metronomic chemotherapy of cyclophosphamide in the drinking water (~10mg/kg/d). Cohort 2 (n=21) is receiving isotype control mAb (100

µg/mouse/injection) in combination with cyclophosphamide (~10mg/kg/d). Cohort 3 (n=32) is receiving no mAb treatment of metronomic chemotherapy. Animals are screened 3 times a week (MWF) for palpable tumors. Antibody injections are given on Tuesdays and Fridays. The mice have been on treatment for over 14 weeks. Palpable tumors have begun to be detected; however at this time there is insufficient data to permit analysis. Tissues have been collected and processed for histological analysis from animals with palpable tumors.

Additional animals are being bred to complete this study as well as for the other experiments outlined in this proposal to determine the immunologic and antiangiogenic mechanisms that underlie the antitumoral effects of combinational targeting of prostate cancer cells, pericytes and endothelial cells with AN-2-specific mAb in combination with metronomic chemotherapy.

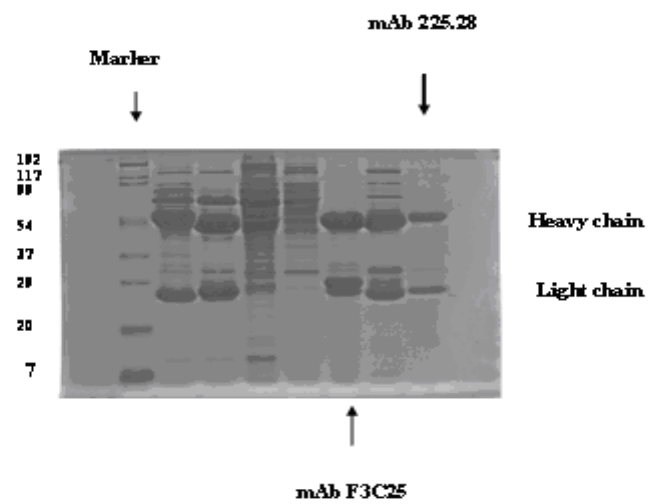


Figure 1. SDS-PAGE analysis under reducing conditions of monoclonal antibodies 225.28 and F3C25 purified from ascitis by sequential precipitation with ammonium sulphate and caprylic acid.

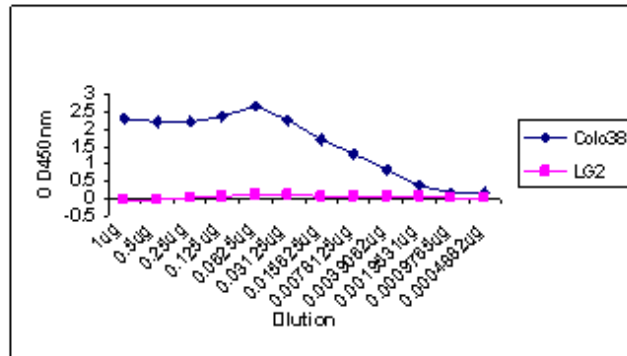


Figure 2- Differential reactivity in ELISA of purified monoclonal antibody 225.28 with the cultured human melanoma cells Colo 38 which express the HMW-MAA and with the cultured human B lymphoid cells LG2, which do not express the HMW-MAA.